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Chemical Enhancement of Viral Transformation in Syrian Hamster Embryo Cells by Gaseous and Volatile Chlorinated Methanes and Ethanes

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Chemical Enhancement of Viral Transformation in Syrian Hamster Embryo Cells by Gaseous and Volatile Chlorinated Methanes and Ethanes[1]

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ABSTRACT

Methods were developed for exposing cells *in vitro* to gases or vapors of volatilized organic liquids. Compounds were selected for their industrial importance, environmental impact, and suspected role in the etiology of some human cancers. Exposure chambers were designed for easy insertion of dishes of cultured cells and were equipped with inlet and outlet ports for introduction and purging of test gases. A gas delivery system utilizing a mass flow meter was used for the quantitative distribution of test gases into exposure chambers. For volatile compounds, appropriate volumes of cold (4°) liquids in glass Petri dishes were quickly placed into chambers, the system sealed, and the compounds rapidly volatilized at 37°. For exposure, the cells and chambers were placed in an incubator and rocked at a constant rate so that a portion of the cells was always in direct contact with the test gases or vapors. Known sample volumes were removed after various treatment times and test gas concentrations determined by standard gas chromatographic techniques. After exposure, the cells were removed and assayed for viability and increased sensitivity to viral transformation. Under these experimental conditions, the volatile liquids 1,1,1-trichloroethane, dichloromethane, chloroform, 1,2-dichloroethane, and 1,1-dichloroethane significantly enhanced transformation of Syrian hamster embryo cells by SA7 adenovirus, while acetone exerted no effect. The gases chloromethane and vinyl chloride were also active in this test system, while bromomethane, methane, and ethane were inactive. Incorporation of some of these compounds into liquid cell culture medium for cell treatment was either unsuccessful or produced only a weak enhancement response. Methodology is now available to evaluate volatile and gaseous carcinogens or mutagens and can be used to identify their mechanisms of action and the relative hazards of these agents to human health.

INTRODUCTION

To prevent human cancers caused by exposure to environmental carcinogens, suitable methodologies and bioassays must be developed that will permit the detection of all classes and physical forms of carcinogenic agents. Quantitative cell culture assay systems provide procedures for detecting potential carcinogens in a relatively short period of time compared to *in vivo* tests. Mammalian cells in culture are particularly appropriate for such assays because they represent an extension of the *in vivo* experimental models, eliminate host factors, afford a means to control test chemical levels, and can be conducted in a controlled environment. Quantitative linear dose-response relationships have been demonstrated for cell transformation assays using fibroblasts from hamster embryo cells (1, 2, 8, 9). The transformation event data are consistent with a one-hit phenomenon indicating a direct cause-and-effect relationship (7, 8).

A short-term *in vitro* assay for carcinogens and mutagens has been developed (2, 4, 5, 8, 13, 15) that determines the ability of various chemical carcinogens to enhance the adenovirus transformation of SHE[3] cells. The viral enhancement assay reflects the capacity of a chemical to damage cellular DNA either directly or indirectly and provides a rapid mammalian cell bioassay system for screening suspected genotoxic environmental chemicals. This bioassay has detected activity for a large number of compounds from a variety of chemical classes, including alcohols and phenols, aliphatic amines, alkyl sulfates and sultones, aromatic amines, aryl halides, carbohydrates and derivatives, hydroxylamines, mycotoxins, polycyclic hydrocarbons, hydrazines, metal salts, steroid hormones, and chlorinated hydrocarbons (2, 3). The last 4 classes are generally negative in standard bacterial (*Salmonella*) assays (21, 22). Data from approximately 105 test performances in 29 different chemical classes have been published for the enhancement of viral transformation assay (2, 3). With that assay as an index of carcinogenicity, 94% of 130 chemicals with known positive or negative activity agreed with their current *in vivo* classifications regarding carcinogenicity.

Volatile or gaseous chlorinated compounds are ubiquitous environmental contaminants. Their use in industrial workplaces is widespread; for example, the annual production of 1,2-dichloroethane is 11.8 billion pounds/year in the United States (17). Small amounts of chloroform and related chemicals can be detected in municipal drinking water supplies (23), and DCM is used in certain food extraction processes (17).

Previous attempts to evaluate the genotoxic potential of TCE and 1,2-dichloroethane by viral enhancement assays with incorporation into liquid cell culture media were unsuccessful or produced only weak or inconsistent enhancement responses. This report documents the successful development of methods for treating mammalian cell cultures with volatile organic liquids and gaseous compounds and measuring the chemical enhancement of DNA viral transformation in hamster embryo cells. The compounds studied were: TCE, DCM, 1,2-dichloroethane, 1,1-

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[3] The abbreviations used are: SHE, Syrian hamster embryo; TCE, 1,1,1-trichloroethane; DCM, dichloromethane; MDM, modified Dulbecco's medium; FBS, fetal bovine serum; TF, transformation frequency.

dichloroethane, chloroform, acetone, vinyl chloride, chloromethane, bromomethane, methane, and ethane.

MATERIALS AND METHODS

Chemicals. Certified analytical grade chloromethane and vinyl chloride were obtained from Matheson Gas, East Rutherford, N. J., bromomethane from Pfalz and Bauer, Stamford, Conn., and methane and ethane from AIRCO, Research Triangle Park, N.C. All these gases were prepared immediately prior to testing. TCE (97%), chloroform (Gold Label), and 1,2-dichloroethane (99%) were obtained from Aldrich Chemical Co., Milwaukee, Wis., and DCM (99%) from Burdick & Jackson Laboratories Inc., Muskegon, Mich.

Cell Cultures. Primary SHE cells were prepared by trypsinization of eviscerated and decapitated embryos (Charles River Laboratories, Boston, Mass.) after 13 to 14 days of gestation. Cells were resuspended in MDM (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated FBS (Sterile Systems, Inc., Logan, Utah) and $NaHCO_3$ (0.22 g/100 ml). Approximately 5×10^6 cells in 5 ml of medium were plated into 60-mm plastic Petri dishes (Lux, Newbury, Calif.) and incubated in a 5% CO_2 atmosphere at 37°. Total cell counts after 3 days ranged from 4 to 6×10^6 cells/plate.

Continuous lines of Vero monkey kidney cells (ATCC:CC1-81) were subcultured weekly in 100-mm plastic Petri dishes. Growth medium consisted of Eagle's minimum essential medium with 10% heat-inactivated FBS.

Virus. Simian adenovirus SA7 was inoculated onto Vero cell cultures in 100-mm dishes at an input multiplicity of 2 to 3 plaque-forming units per cell. After the virus had been adsorbed for 2 hr, 5 ml of medium were added to each plate. Cytopathic effects were usually complete by 72 hr, after which the infected cells were harvested and then frozen and thawed 4 times, and the virus was separated from cell debris by low-speed centrifugation. Virus stocks for transformation assays were stored in 1- or 2-ml aliquots at −65°.

Transformation Assays. Complete adenovirus transformation procedures have been presented elsewhere (4, 5). SA7 was added to SHE cells (3 to 4×10^7 plaque-forming units per culture) and adsorbed for 3 hr; the virus-inoculated cells were removed with 0.25% trypsin in MDM with 0.1 mM $CaCl_2$, centrifuged, and resuspended to 10^6 cells/ml in MDM with 10% FBS and $NaHCO_3$ (0.11 g/100 ml). The cells were mixed vigorously and plated into 60-mm dishes at 2×10^5 cells/dish; 3 ml of the above medium was then added to each plate. After incubation for 3 days, the medium was changed to MDM with 0.1 mM $CaCl_2$ (10), 10% FBS, and $NaHCO_3$ (0.22 g/100 ml). After 6 days, transformation assay plates were overlaid with 3 ml of the above medium containing Bactoagar (BBL Microbiology Systems, Cockeysville, Md.) (0.3 g/100 ml). At intervals of 4, 5, and 6 days, 3 ml of additional agar medium were added. Final focus counts were made 25 to 30 days from the beginning of the experiment.

Survival Assays. After resuspension of the SHE cells to 10^6 cells/ml (see "Transformation Assays"), the cells were diluted 1:300 in medium to give ~333 cells/0.1 ml. Then 0.2 ml (~666 cells) was added to each of 5 plastic dishes, followed by 3 ml of MDM with 10% FBS and $NaHCO_3$ (0.11 g/100 ml). Five to 6 days later, 3 ml of medium with double the amount of $NaHCO_3$ was added to each plate, and after 8 to 9 days of total incubation the cell colonies were fixed in 10% buffered formalin and stained with 0.02% crystal violet (Fisher Scientific, Raleigh, N. C.). The cloning efficiencies of virus-inoculated cells under these conditions were usually 8 to 15%.

Determination of Enhancement. The fraction of cells surviving treatment was determined from assay plates which were seeded with ~666 cells/plate. The number of colonies in 5 plates seeded with treated cells was divided by the number of colonies in 5 plates with control cells to give the surviving fraction of treated cells.

The total number of SA7 foci in 10 control plates, each receiving 2×10^5 cells, was used as the frequency of transformation per 2×10^6 virus-inoculated control cells. The TF of treated cells (number of SA7 foci per 10^6 surviving cells) was calculated by dividing the number of SA7 foci from 10 plates by the surviving fraction of treated cells. Detailed methods for determining the frequency of transformation and enhancement ratios using data from actual enhancement experiments have been published (4, 6). Similar methods for calculation have been used for the determination of mutation frequency and for the demonstration of enhancement of SV40 transformation by DNA base analogues (29) in mammalian cells.

Enhancement was expressed as the ratio between the TF of treated, surviving cells and the TF of control cells. Statistical significance was determined using a table of ratios (4) derived from the Lorenz table (20) that is based upon the Poisson distribution. The increased TF was considered statistically significant at the 5 or 1% confidence level if the enhancement ratio exceeded the appropriate value obtained from the Lorenz table.

Exposure of SHE Cells to Gases and Volatile Compounds. Polycarbonate treatment chambers (4.6-liter) (Billups-Rothenberg, Flow Laboratories, McLean, Va.) were equipped with inlet and outlet ports for easy introduction and purging of gases. The chambers could be opened for insertion of dishes of cultured cells and then resealed with steel spring clamps. After exposure, the gases or vapors were exhausted through charcoal under negative pressure. Before reuse of test chambers, any residual chemical was removed by heating at 100° under negative pressure. Individual chambers were used for exposure to each concentration of volatile liquid or gas.

Cells were cultured in 60-mm glass Petri dishes (Corning Glass, Corning, N. Y.). Fresh medium was added to the cells 8 to 24 hr prior to treatment; this medium was replaced with 1 ml of medium supplemented with 7.5 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (Leon Labs, St. Louis, Mo.) buffer in place of sodium bicarbonate. Ten ml of sterile water were added to the bottom of each chamber to ensure adequate humidification and to prevent desiccation of the small volumes of cell culture medium.

When volatile liquids were tested, the chemicals, pipets, and Petri dishes were prechilled to −20°. Appropriate volumes of volatile compounds were mechanically dispensed with a glass pipet into an open glass Petri dish. Dishes containing the test chemicals were quickly placed into the exposure chambers containing dishes of cells at 37°, and the chambers were rapidly sealed. Depending on the density of the test chemical vapor relative to air, the dish containing the chemical was placed above or below the test cells. Most volumes (generally 0.05 to 2.0 ml) of test compounds were completely volatilized within 10 to 30 min at 37°.

A gas delivery system with a mass flow meter (Kurz Instruments, Carmel Valley, Calif.) was used for quantitative distribution of test gases to the exposure chambers. Oxygen and nitrogen were dispensed and metered through standard regulators and rotameters (Fischer and Porter, Warminster, Pa). The accuracy of all flow rates was confirmed by use of a standardized bubble chamber. Test chambers were purged of residual air with 5 changes of test gas that had been documented to give an approximately 99% accurate atmosphere concentration. To confirm the accurate dilution and delivery of calculated gas concentrations by this system, nitrogen oxide was dispensed similarly, and a nitrogen oxide analyzer was used to determine the actual gas concentrations.

Following introduction of chemicals, the sealed chambers and cells were placed onto a rocker platform (Bellco Glass, Inc., Vineland, N. J.) at 37°. The chambers were tilted to provide a sufficient inclination (10°) and rocked 4 times per min so that a portion of the cell monolayer was always in contact with the test gas or vapor. Treatment times ranged from 2 to 20 hr, after which the gases were exhausted and the cells assayed for viability and enhancement of viral transformation.

Analytical Determinations. Samples of chamber atmospheres were obtained and analyzed on a Hewlett-Packard 5840 gas chromatograph with flame ionization detection and a capillary inlet system (Hewlett-Packard Co., Palo Alto, Calif.). The gas chromatograph oven was isothermal at 50 to 70°; the injector temperature was 300°; and the detector temperature was 325°. The sample gases were carried on helium at a

rate of 1.2 cu cm/min through the 26-m SE-54 fused silica capillary column.

RESULTS

Five 2-fold serial dilutions of 1,2-dichloroethane and TCE were tested for cytotoxicity and enhancement of viral transformation in the SHE cell system. Table 1 shows the results of exposure to TCE and 1,2-dichloroethane added to liquid cell culture media for approximately 20 hr. Under these conditions, with treatment doses as high as 500 or 1000 μg/ml, respectively, neither compound was overly toxic to mass cultures of SHE cells as demonstrated by subsequent cloning assays for viability. TCE reduced cell viability (31% survival) only at the highest concentration tested. Both 1,2-dichloroethane and TCE are immiscible in cell culture medium, and at concentrations greater than 250 μg/ml they separate out and form globules at the bottom of the Petri dish. Cells exposed to DCE and TCE by this treatment protocol produced no increased enhancement of transformation by SA7 at any dose level; no SA7 foci were observed at 1000 μg/ml of TCE, presumably due to the high toxicity.

Tables 2 and 3 show the effects of pretreatment of mass cultures of hamster embryo cells with the vapor phase of 5 volatile and 5 gaseous methanes and ethanes for 2 to 20 hr. Preliminary experiments indicated that treatment with chloroform for 3 hr was optimal. Five 2-fold chemical or gas dilutions, extending over approximately a 20-fold dose range, were tested for all compounds. Dose ranges were selected from preliminary toxicity screens (data not shown). For brevity, some 100% lethal dose levels have been omitted. All compounds were tested from 100% lethal to nonlethal doses, which are generally found within a narrow range (Tables 2 and 3).

Five volatile chlorinated methanes and ethanes (DCM, chloroform, 1,2-dichloroethane, 1,1-dichloroethane, and TCE) significantly enhanced the viral transformation frequency in a dose-dependent manner. An enhanced frequency of transformation was shown for at least 2 or more dose levels tested. All the chlorinated organics except for chloroform produced a significant absolute increase in the number of viral foci at several doses. Therefore, increased enhancement ratios were not dependent upon cell killing for statistical significance. The commercial preparation of TCE tested was stabilized with 3% 1,4-dioxane (manufacturer's specifications), itself a suspected cancer agent (16). However, other samples of TCE not containing 1,4-dioxane were similarly as active (data not shown). These results indicate that TCE, not the stabilizer, was responsible for the enhancement

Table 1

Effect of 20-hr pretreatment of SHE cells with 1,2-dichloroethane or TCE on Transformation by SA7: exposure of cells to chemical in culture medium

Chemical[a]	Final concentration (μg/ml)	Surviving fraction[b]	SA7 foci[c]	Enhancement ratio[d]
1,2-Dichloroethane	1000	0.90	26	1.0
	500	1.04	31	1.1
	250	1.00	37	1.3
	125	0.98	24	0.9
	62	1.00	30	1.0
	0	1.00	29	1.0
	A[e]	1.01	21	0.7
TCE	1000	0.31	0	0
	500	1.06	49	0.9
	250	0.96	43	0.9
	125	1.04	35	0.7
	62	0.99	36	0.7
	0	1.00	49	1.0
	D	0.96	42	0.9

[a] Chemical dilutions in culture medium were added to mass cultures of SHE cells 20 hr before SA7 addition. Virus was adsorbed for 3 hr, and the cells were transferred for survival (500 to 700 cells/dish) and for transformation assays (200,000 to 300,000 cells/dish).

[b] Determined from plates receiving 500 to 700 cells. The number of colonies from virus- and chemically treated cells was divided by the number of colonies from virus-inoculated control cells to give the surviving fraction. Cloning efficiencies of control cells treated with virus were from 8 to 15%.

[c] Number of foci from 10^6 plated cells.

[d] Enhancement ratio was determined by dividing the TF of treated cells (TF = SA7 foci × reciprocal of the surviving fraction) by that obtained from control cells.

[e] Solvent controls: A, acetone; D, dimethyl sulfoxide at a final concentration of 0.5%.

Table 2

Enhancement of viral transformation in SHE cells by pretreatment with volatilized chlorinated methanes and ethanes in sealed chambers

Chemical[a]	Treatment dose[b] (ml/chamber[c])	Surviving fraction[d]	SA7 foci[e]	Enhancement ratio[f]
DCM	5.0	0	0	NA[g]
	2.5	0.26	19	2.6[h]
	1.25	0.60	50	3.0[h]
	0.62	0.93	44	1.7[i]
	0.31	1.22	39	1.1
	0	1.00	28	1.0
Chloroform	2.0	0	0	NA
	1.0	0	0	NA
	0.50	0.20	43	4.6[h]
	0.25	0.49	40	1.7[i]
	0.12	0.86	29	0.7
	0	1.00	47	1.0
1,1-Dichloroethane	1.0	0	0	NA
	0.50	0.98	164	1.4[i]
	0.25	0.83	194	1.9[h]
	0.125	0.72	189	2.2[h]
	0.062	0.87	163	1.5[h]
	0	1.00	122	1.0
1,2-Dichloroethane	0.80	0	0	NA
	0.40	0.78	197	2.5[h]
	0.20	0.64	283	4.3[h]
	0	1.00	103	1.0
TCE	1.0	0	0	NA
	0.50	0.41	57	1.5[i]
	0.25	0.63	146	2.5[h]
	0.125	0.95	137	1.6[h]
	0.062	1.47	103	0.8
	0	1.00	92	1.0
Acetone	1.6	0	0	NA
	0.80	0.66	67	0.9
	0.40	0.75	85	1.0
	0.20	0.61	66	1.0
	0.10	0.84	102	1.1
	0	1.00	109	1.0

[a] Mass cultures of hamster embryo cells were treated for approximately 20 hr with volatilized liquids (3 hr for chloroform) before addition of SA7. Virus was adsorbed for 3 hr, and the cells were transferred for survival (500 to 700 cells/dish) and transformation assays (200,000 to 300,000 cells/dish).

[b] See Table 4 for the actual amounts of volatilized liquid vapors present in the treatment chamber atmosphere as determined by gas chromatography.

[c] ml of chemical added per 4.6-liter chamber.

[d] Determined from plates receiving 500 to 700 cells. The number of colonies from virally and chemically treated cells was divided by the number of colonies from virus-inoculated control cells to give the surviving fraction. Cloning efficiency of control cells treated with virus was from 8 to 15%.

[e] Number of foci from 2×10^6 plated cells.

[f] Enhancement ratio was determined by dividing the TF of treated cells (TF = SA7 foci × reciprocal of the surviving fraction) by that obtained from control cells.

[g] NA, not applicable.

[h] Statistically significant at the 1% level.

[i] Statistically significant at the 5% level.

Table 3

Enhancement of viral transformation in SHE cells by pretreatment with gaseous methanes and ethanes in sealed chambers

Chemical[a]	Treatment dose (parts/10^3)	Surviving fraction[b]	SA7 foci[c]	Enhancement ratio[d]
Vinyl chloride	160	0.93	80	1.6[e]
	80	0.86	102	2.2[e]
	40	1.01	76	1.4
	20	0.89	86	1.8[e]
	0	1.00	55	1.0
Chloromethane	50	0	0	NA[f]
	25	0.12	64	8.3[e]
	13	0.80	143	2.8[e]
	6	0.86	134	2.4[e]
	3	0.89	86	1.5
	0	1.00	64	1.0
Bromomethane	8	0.87	71	1.3
	4	1.05	63	1.0
	2	0.97	50	0.8
	1	1.30	49	0.6
	0	1.00	61	1.0
Methane	800	1.02	30	0.8
	400	1.11	36	0.8
	200	0.94	27	0.7
	100	1.04	43	1.1
	50	0.98	24	0.6
	0	1.00	39	1.0
Ethane	800	0.84	33	1.0
	400	0.98	50	1.3
	200	1.12	36	0.8
	100	0.96	26	0.7
	50	0.89	21	0.6
	0	1.00	39	1.0

[a] Mass cultures of hamster embryo cells were treated for approximately 20 hr (2 hr for methane and ethane) with volatilized liquids before addition of SA7. Virus was adsorbed for 3 hr, and the cells were transferred for survival (500 to 700 cells/dish) and transformation assays (200,000 to 300,000 cells/dish).

[b] Determined from plates receiving 500 to 700 cells. The number of colonies from virally and chemically treated cells was divided by the number of colonies from virus-inoculated control cells to give the surviving fraction. Cloning efficiency of control cells treated with virus was from 8 to 15%.

[c] Number of foci from 2×10^5 plated cells.

[d] Enhancement ratio was determined by dividing the TF of treated cells (TF = SA7 foci × reciprocal of the surviving fraction) by that obtained from control cells.

[e] Statistically significant at the 1% level.

[f] NA, not applicable.

response. A noncarcinogenic, nonchlorinated volatile solvent control, acetone, was tested from lethal to nonlethal concentrations and produced no enhancement of viral transformation (Table 2).

The gases vinyl chloride, chloromethane, bromomethane, methane, and ethane were evaluated in the viral enhancement assay by exposing SHE cells for 2 to 20 hr. Both vinyl chloride and chloromethane demonstrated significant responses in this system after 20 hr of exposure. Bromomethane, methane, and ethane were inactive at the concentrations utilized after 2 or 20 hr of exposure.

The concentrations of several of the chlorinated chemicals present in sealed treatment chambers at different time intervals were determined by standard gas chromatographic techniques (Table 4). Triplicate samples from individual chambers with 3 different volumes of DCM showed approximately proportional levels in detectable DCM after 2 hr. Concentrations of DCM detected at 2 hr were approximately 20% of calculated theoretical values, and decreased to approximately 10% after 20 to 24 hr. The concentration of TCE detected at 2 hr was related to the volume of TCE initially volatilized in the treatment chamber and was, as with DCM, a fraction of calculated theoretical values. TCE values did not significantly decrease over a 24-hr period. A 2-hr time period was selected as the initial sampling period because this was the minimum time necessary for equilibration of the system and it produced maximal values. Samples taken earlier (data not shown) showed low values and were not reproducible or consistent.

DISCUSSION

Volatile liquids and gases constitute a significant portion of our potential carcinogenic hazards. For example, many of the chemicals used in the industrial workplace are gases or compounds that may volatilize under ambient temperatures. Petrochemical refining and energy-generating plants produce large amounts of gaseous pollutants (26). Some gases and organic liquids are slightly soluble in water and may contaminate municipal drinking water supplies (23).

Table 4

Concentrations of volatilized chlorinated compounds present in treatment chamber atmospheres as determined by gas chromatography

Chemical	Chemical volatilized/chamber (ml)	Theoretical concentration (μg/cu cm)	Concentration (gas chromatography) (μg/cu cm)		
			2–4 hr	12 hr	20–24 hr
TCE	2.00	540	58	ND[a]	59
	1.00	270	25	7	10
	0.50	134	26	27	23
	0.25	67	19	14	15
	0.12	32	11	ND	8
	0	0	0	0	0
DCM	2.50	670	120	ND	67
	1.25	330	73	ND	17
	0.63	170	30	13	12
	0	0	≤2	≤2	≤2
1,2-Dichloroethane	0.75	190	14	2	6
	0.38	94	4	ND	2
	0.19	47	3	2	2
	0.10	24	3	<1	≤1
	0.05	12	<1	ND	≤1
	0	0	0	ND	≤1

[a] ND, not determined.

Closed treatment chambers have been utilized by others for treatment of prokaryotic or eukaryotic cell systems with volatilized liquids or gases. Cultured cells treated in this manner have subsequently been assayed for toxicity or mutation (11, 18, 19, 25–28). However, previous attempts to assay for transformational events following this type of treatment have not been published.

A sensitive and well-validated bioassay (enhancement of viral transformation in hamster cells) that closely correlates with *in vivo* carcinogenesis tests was selected as an indicator system for developing and applying methods appropriate for testing volatile liquids and gases. The treatment procedures described increased the sensitivity of hamster embryo cells to viral transformation. Additionally, the treatment resulted in the consistent and reproducible detection of some compounds that previously produced weak or no enhancement responses when incorporated into liquid cell culture media.

Concentrations of chemicals detected by gas chromatographic analysis after relatively short volatilization times (2 hr) were related to the volumes of chemical added. However, analytical determinations confirmed that the concentrations of gaseous chemicals decreased with time in the sealed treatment chambers; this was not unexpected. Absorption and/or penetration of vapors or gases into cells, media, and the treatment chamber walls seem the most likely explanation and are consistent with results reported by others (18, 19).

Although small volumes of added chemicals produce low concentrations that approach the limits of detectability by gas chromatography, and larger volumes saturate the treatment chamber, the volumes needed to produce cell killing and/or enhanced viral transformation can be accurately determined during the initial treatment period. It is evident that the concentrations of some compounds do not remain constant for the entire 20- to 24-hr treatment period. Therefore, some chemicals may need to be readded to treatment chambers if first results are negative.

The technical problems involved in determining accurately the amount of chemical interacting with critical cellular target macromolecules (*i.e.*, DNA) are considerable and need not be addressed for preliminary screening assays. The approach taken in these studies was to use a range of exposure concentrations and confirm the interaction of test chemical with cellular macromolecules by assaying for the ability of the cells to attach and divide.

The positive effects observed (*i.e.*, enhanced viral transformation) in cultured cells treated with the volatile liquids or gases are in agreement with their positive carcinogenic response (*i.e.*, tumor formation) observed in rodents treated with these same chlorinated compounds. These latter responses have been summarized by Infante and Tsongas (17) from ongoing experiments with animals exposed to chloroethanes and chloromethanes from the National Cancer Institute/National Toxicology Program Bioassay program and from privately supported industrial studies. 1,2-Dichloroethane and chloroform produced tumors in several tissue sites of both sexes of mice and rats treated by gavage. Male rats treated by inhalation with dichloromethane developed salivary gland tumors. Male mice similarly exposed to chloromethane had kidney tumors. Animal bioassays (National Cancer Institute/National Toxicology Program) have been initiated with TCE, but no data are available at this time. However, TCE was shown to transform cultured rat embryo cells, if treated cells were passaged a sufficient number of times following exposure (24). Its close structural analogue, 1,1,2-trichloroethane, given by gavage has produced liver and adrenal gland tumors in male and female mice.

In summary, a method suitable for the bioassay of diverse volatile liquids and gases in a well-validated mammalian embryo cell system has been developed. Results of tests with a series of chlorinated compounds of industrial and environmental importance document that this system is sensitive and quantitative. The relative ease and simplicity of the treatment protocol described should allow detection and the study of biological mechanisms of these types of chemicals in other validated *in vitro* bioassay systems for carcinogenesis and mutagenesis. Along these lines, we have recently reported the genetic activity of formaldehyde (12) and ethylene oxide (14) in mammalian cells.

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